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The Action of a Dietary Retinoid on Gene Expression and Cancer Induction in Electron-irradiated Rat Skin

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Current models of radiation carcinogenesis generally assume that the DNA is damaged in a variety of ways by the radiation and that subsequent cell divisions contribute to the conversion of the damage to heritable mutations. Cancer may seem complex and intractable, but its complexity provides multiple opportunities for preventive interventions. Mitotic inhibitors are among the strongest cancer preventive agents, not only slowing the growth rate of preneoplasias but also increasing the fidelity of DNA repair processes. Ionizing radiation, including electrons, is a strong inducer of cancer in rat skin, and dietary retinoids have shown potent cancer preventive activity in the same system. A non-toxic dietary dose of retinyl acetate altered gene expression levels 24 hours after electron irradiation of rat skin. Of the 8740 genes on an Affymetrix rat expression array, the radiation significantly (5 fold or higher) altered 188, while the retinoid altered 231, including 16 radiation-altered genes that were reversely altered. While radiation strongly affected the expression of stress response, immune/inflammation and nucleic acid metabolism genes, the retinoid most strongly affected proliferation-related genes, including some significant reversals, such as, keratin 14, retinol binding protein, and calcium binding proteins. These results point to reversal of proliferation-relevant genes as a likely basis for the anti-radiogenic effects of dietary retinyl acetate.

INTRODUCTION

Current models of radiation carcinogenesis generally assume that the DNA is damaged in a variety of ways by the radiation and that subsequent cell divisions contribute to the conversion of the damage to heritable mutations. Typically full malignancy is a multistage process, but even a single mutation may sometimes be sufficient to produce a benign precancerous lesion that exhibits increased proliferative capability, possibly because of the mutation[1)]. The riskiness of proliferation for a cell with damaged DNA is implicit in the temporary blockage of proliferation as the first step in p53-mediated DNA excision repair pathways[2)].

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Within the context of this general model, cancer preventive agents may be acting at several possible vulnerable steps. For example, evidence indicates that slowing the growth of the precancerous lesions can reduce the incidence of malignancies[2–5)]. Perhaps the diversity of the carcinogenic process is why known cancer preventive agents differ so widely, including such disparate agents as: organ-specific stimulants, DNA repair-enhancers, radical-trapping agents, nutritional anti-oxidants, and cytokines. Experiments have established that varying percentages of experimental cancers may be prevented by surprisingly non-toxic, dietary agents[6)].

Radiation, like most carcinogens, causes cell death, chromosomal breaks, generation of reactive oxygen moieties, followed by a complex array of cellular responses, including temporary cell cycle arrest, apoptosis, hyperplasia and cancer induction[7,8)]. Radiation-induced mutations can activate oncogenes and inactivate tumor suppressor genes, which provides a possible direct route whereby radiation causes cancer. For example, K-*ras* and/or c-*myc* oncogenes were activated in all tested rat skin tumors induced by low-LET

radiation[7], and amplification of oncogenes, such as c-*jun* and c-*fos*, in skin cancers have been reported elsewhere[9,10].

The differentiation pattern of human epidermal keratinocytes can be altered profoundly by retinoids[11]. Retinoids exert their activity by modulating gene expression following interaction with and activation of the nuclear retinoic acid receptors and transcription factors RAR and RXR[12], each receptor has three isoforms: α, β and γ making retinoid signaling highly complex since multiple regulation pathways are involved.

Retinoids have considerable anti-tumor activity against a variety of tumors, including mammary, head and neck, and skin tumors, and they have been put into clinical use for chemoprevention of various tumors[13]. But the mechanism of how retinoids inhibit tumor formation is not clear. Retinoids might be inducing apoptosis or acting as antioxidants[14,15], or inducing differentiation leading to cell death[16–18]. It is known that human cells respond to x-ray with inducible early, intermediate, and late responses at the transcriptional level[19].

There are many reports on radiation inducible genes in mammalian cells or tissues, such as *P21 WAF1/Cip1*[20], c-*MYC*, *NEUROLEUKIN*, Co.Zn-*SOD*, *BCL-2*[21], etc. Some other genes are reported to be down-regulated by radiation in other cell lines or tissues, such as DNA. *LIGASE I*, *CD19*[22], *CYCLIN B*[23], *STATHMIN*[24], c-*MYC*, and *TOP II*[25]. Generally radiation-induced gene expression varies widely in cell lines of different origin and in different tissues, which implies that cell type is an important determinate of radiation response.

Previous work has showed that 50% of the rat skin cancers induced by single doses of low-LET radiation could be prevented by a dietary supplement of 300 ppm retinyl acetate beginning 2 weeks prior to radiation and ending 1 week after the radiation[26]. This result suggests that vitamin A can inhibit cancer-relevant event(s) occurring at about the time of irradiation[26].

MATERIALS AND METHODS

Eight 23 day old rats were divided into 2 dietary groups of 4 rats each: Group 1 was fed with regular lab chow, Group 2 was fed chow supplemented with 300 ppm vitamin A acetate (retinyl acetate). One week later, the rats were exposed to carcinogenic skin surface doses of 9 Gy or 18 Gy of electron radiation, on 2 separate 1.5 cm × 2 cm dorsal skin areas; a third unexposed area served as control tissue.

Twenty-four hours later, selected as an intermediate point between short and long-term gene expression changes, the rats were euthanized, and squares 7 mm on a side were excised from the center of each area. Total RNA was extracted from these samples with TriZol reagent (Gibco BRL, NY) and like-treated samples from different rats were pooled. The cRNAs were differentially displayed by means of rat genome gene chips (RAT U34-A) on a commercial chip reading system (Affymetrix, CA). The expression of all 8740 rat genes (about 90% known) was examined in single hybridizations. Based on raw signals above 1000 flourescence units (corresponding to about 60% 'present' calls), 4402 of the 8740 (50.4%) genes showed positive expression in at least one of the 5 treatment groups.

RESULTS AND DISCUSSION

Based on genes with ≥5 fold (induction or inhibition) expression changes, the treatments were positive relative to control as follows: 1) 9 Gy showed 188 altered genes; 2) 18 Gy showed 92 altered genes, including 54 genes overlapping genes with 9 Gy; 3) vitamin A showed 231 altered genes; and 4) 9 Gy radiation + vitamin A vs. 9 Gy showed 58 altered genes. Of special interest were genes that were reversely regulated by vitamin A and radiation. There were 15 genes that showed at least a 5 fold increase by radiation and a 2.0 fold reversal with vitamin A. Among the genes that showed the reversal were: keratin 14 (K14), retinol binding protein II, 2 intracellular calcium-binding proteins, and beta defensin 2 (Table 1).

When expressed genes were grouped according to function, several categories failed to show any changes after irradiation with or without vitamin, including apoptosis-related genes and oncogene/tumor suppressor genes. Of 16 cytokines only one (Cyclin G) showed >2 fold change between 9 Gy and vitamin A + 9 Gy. Figure 1 shows how the 100 most strongly altered genes were distributed according to treatment and biological function. Compared to estimates based on randomly selected genes, the percentage of genes affected by 9 Gy or vitamin A or both was higher for epidermal differentiation genes and lower for nucleic acid metabolism genes. Cytoskeleton/matrix/adhesion and stress response genes showed vitamin A reversal of some radiation-induced inductions. Cellular metabolism genes were increased by vitamin A irrespective of radiation, suggesting vitamin A stimulated some cellular activities and protein processes, and mobilized a greater number of cellular metabolism enzymes in comparison to no treatment. Genes in the immune/inflammation category showed induction by radiation irrespective of vitamin A treatment, confirming that radiation causes inflammation, but vitamin A could not

Table 1 Expression levels (normalized fluorescence units) of radiation-induced genes most strongly reversed by or additive with dietary vitamin A

Control	vitamin A*	9 Gy*	9 Gy+ vit. A*	18 Gy*	Gene Description
				Reversal Responses	
4758	4162	55945	8190	21710	D63774 RATRETK rat n. mRNA for keratin 14
42	593	18930	1250	24441	L18948 intracellular calcium-binding protein (MRP14)
0	0	4060	0	4038	AF068861 rat n. beta defensin-2
1413	2424	31193	14018	25887	clone rx04836, homologous to mouse SCC antigen 2
1369	2052	22428	11509	11433	M13949 cellular retinol binding protein II (CRBPII)
5817	3764	21478	4049	25728	rc_AA957003, homologous to calcium-binding protein 8
				Additive Responses	
1848	5388	3232	8916	3317	L28818cds RATINVO rat n. involucrin gene
4037	1990	3700	1228	4569	J04792 ornithine decarboxylase (ODC) gene

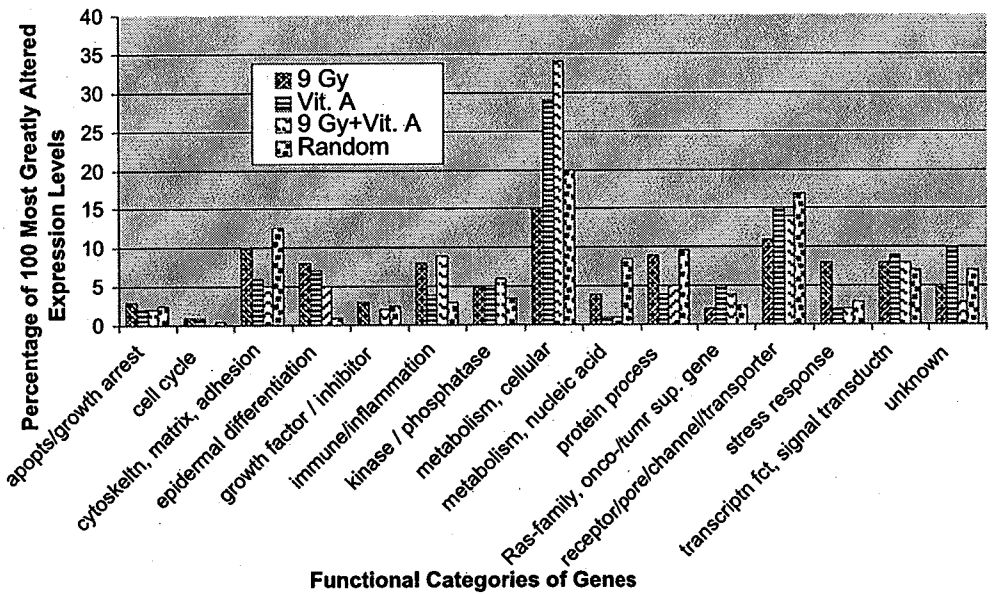

Fig. 1. Comparison of the genes affected by radiation and Vitamin A sorted according to functional categories. The 100 most strongly altered genes were assigned to the indicated functional categories for treatment groups : 1) 9 Gy, 2) Vitamin A and 3) 9 Gy plus Vitamin A. A category distribution in untreated controls (labeled 'random' in the figure) was generated by randomly selecting 200 genes from the 4402 rated "present" by Affymetrix software and placing them in the appropriate categories.

reverse these effects. These results point to reversal of proliferation-relevant genes as a possible basis for the anti-radiogenic effects of dietary retinyl acetate.

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